

JACOB'S MODEL - PAPER II

# AUTISM AFTER RUBELLA

Human Evidence and a Testable Neurodevelopmental Mechanism

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Jacob's Voice

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Jacob would like to give credit to God for his amazing abilities.

## Scope

This paper investigates Rubella virus (RUBV) as an independent biological entity. Its starting point is a human observation that does not require mechanistic inference: autism was documented longitudinally in children with congenital Rubella. The paper does not propose that Rubella explains autism generally, and it does not infer causation from vaccination history, antibody titer, or diagnosis alone. It asks whether the congenital human precedent, together with modern RUBV neurodevelopmental biology, supports testing for a biologically defined RUBV-associated autism subtype outside congenital infection.

## AUTHOR'S NOTE - ORIGIN AND GOVERNANCE OF THE MODEL

The mechanistic model developed in this paper originated with Jacob Boatman. Jacob identified Rubella virus as the central biological focus and proposed that lysosomal function, intracellular cholesterol availability, glial signaling, and host susceptibility could contribute to an autism-associated phenotype in a subset of individuals. Scott Boatman worked with Jacob to translate these ideas into experimentally defined questions and investigate them against the published scientific literature.

The cited literature establishes the individual observations and mechanistic precedents. Their integration into the specific hypothesis presented here is Jacob's model. Evidence controls the language: demonstrated findings are stated directly; mechanistic bridges remain labeled as bridges; and missing RUBV-specific arrows are not filled by inference.

## Relationship to the Foundational Thesis

Paper I places the leading endolysosomal bottleneck downstream of substantial LAL hydrolysis, at NPC2-mediated free-cholesterol availability and transfer. Paper II asks whether that cellular phenotype, if demonstrated during RUBV infection, could contribute to neurodevelopmental pathology alongside an independently testable glial/interferon route.

## Abstract

The relationship between congenital Rubella and autism is a documented human finding, not a theoretical extrapolation. In Stella Chess's longitudinal cohort of 243 children with congenital Rubella, 7% were diagnosed with autism during the preschool period. Follow-up documented both remission of earlier diagnoses and newly appearing cases, and Chess interpreted Rubella as etiologic in that congenital cohort. The strongest conclusion supported by those data is direct: congenital RUBV infection can be followed by a human neurodevelopmental syndrome that includes autism. This establishes a human etiologic precedent under fetal infection. It does not establish that postnatal RUBV infection or vaccination causes autism.

Modern human neural studies make that historical finding biologically more specific. RUBV predominantly infects microglia in human fetal brain tissue and microglia-containing organoids while producing strong interferon responses in neighboring neurons and neural progenitor cells. Earlier human fetal glial studies independently demonstrated productive infection, with astrocytes highly permissive. Thus the congenital autism record is now accompanied by direct evidence that RUBV can enter and perturb the cellular environment of the developing human brain.

A second, experimentally separable route arises from RUBV endolysosomal biology. RUBV replication occurs in virus-modified lysosomes, P150N physically interacts with ARL8A, and host ARL8/HOPS/CI-M6PR biology directly controls lysosomal NPC2 availability and cholesterol egress. The current Jacob model predicts a post-LAL NPC2 bottleneck: cholesteryl-ester hydrolysis remains substantially intact and free cholesterol is generated, but NPC2 availability or transfer becomes rate-limiting, increasing late-endosomal/lysosomal unesterified-cholesterol residence and reducing downstream ER availability. This RUBV-specific functional chain has not yet been demonstrated.

Timing is a central part of the model. RUBV replication compartments change structurally over time: early replication structures mature into larger endolysosomal networks, with major remodeling documented across later infection states. Much of the functional RUBV literature interrogates entry or early infection, or uses windows that do not directly resolve the mature P150/dsRNA-positive compartment. The proposed NPC2 phenotype is therefore assigned to established and late compartment biology. An early negative assay does not test that late-stage prediction unless the same pathway is measured across maturation.

Paper II therefore advances a strong but bounded disease-level hypothesis: RUBV has already been linked to an autistic phenotype under congenital human infection; the unresolved question is whether a mechanistically related RUBV-associated autism subtype also exists outside congenital infection in a susceptible biological subset. That subtype should be claimed only if prospectively defined virological, cellular, temporal, and neurodevelopmental evidence converges in the same individuals.

## 1. Central Question

The congenital question is not hypothetical: autism has already been documented in a longitudinal human congenital-Rubella cohort. The open question is whether a related RUBV-associated neurodevelopmental subtype exists outside congenital infection in a biologically identifiable subset.

This hypothesis predicts heterogeneity, not universality. It does not require RUBV to explain autism broadly. It predicts that, if a non-congenital RUBV-associated subtype exists, it should be identifiable by convergent biology rather than by autism diagnosis, vaccination history, Rubella IgG titer, serum cholesterol, or any single laboratory marker.

## 2. Human Precedent: Congenital Rubella

Chess followed 243 children with congenital Rubella. At preschool age, 37% had intellectual disability, 15% had reactive behavior disorder, and 7% had autism; among 205 children reassessed around ages 8-9, two earlier autism diagnoses remitted and three new cases appeared [1-3]. Chess interpreted Rubella as an etiologic agent in that congenital cohort [2]. The infection necessarily preceded the later behavioral diagnosis, and the cohort was followed longitudinally rather than assembled from children selected for autism.

This is direct human evidence, not a mechanistic analogy and not a comparison to an unrelated disorder. Autism was part of the observed neurodevelopmental outcome after congenital RUBV infection. Historical diagnostic criteria and substantial sensory, neurological, and intellectual comorbidity limit how precisely those cases map onto current ASD definitions, but they do not erase the reported autistic syndrome or Chess's etiologic interpretation.

The evidence therefore supports a stronger conclusion than simple association language implies: congenital RUBV is a demonstrated human setting in which an autistic phenotype can emerge during neurodevelopment. What remains unproven is whether postnatal RUBV infection, persistent RUBV, or Rubella vaccination can produce a related phenotype.

## 3. Developing-Brain Biology

In 2023, Popova and colleagues studied human fetal brain slices and microglia-containing organoids. RUBV predominantly infected microglia, while infection produced a strong interferon response in non-microglial cells, including neurons and neural progenitor cells [4]. This places RUBV directly inside a developing-human-brain system and shows that productive infection of one neural-lineage population can alter signaling in neighboring developmental cell types without requiring widespread neuronal infection.

Earlier human fetal glial work independently demonstrated productive RUBV infection, with astrocytes representing a major permissive population [5]. Together, these human studies establish that RUBV has direct access to glial populations capable of shaping neural development. The remaining question is not whether RUBV can act in developing human neural tissue; it can. The unresolved issue is whether this biology contributes to autism outside congenital Rubella.

## 4. Persistence and Host Susceptibility

Persistence also makes late compartment biology experimentally relevant. Classic RUBV morphology supports outward traffic from late virus-modified endolysosomal structures, interpreted as plasma-membrane fusion and expulsion of lysosomal by-products. No direct RUBV experiment has quantified extracellular NPC2, LAL, SMPD1, FUCA1, or surface LAMP1 as a lysosomal-exocytosis readout. Reduced cargo delivery plus increased outward loss is therefore a two-hit prediction, not a demonstrated mechanism.

Wild-type clinical RUBV can establish persistent productive infection in primary human fetal endothelial cells with little cytopathology and continued release of infectious virus through serial passages [6]. Separately, infectious RA27/3-derived RUBV has been recovered from granulomas of patients with primary immunodeficiency, and viral divergence correlates with persistence duration, demonstrating intrahost

evolution [7]. These findings prove persistence is biologically possible in specific contexts. They do not demonstrate routine persistence after vaccination or persistence in autistic children. The proposed variable is therefore not exposure alone but RUBV multiplied by host susceptibility. Prolonged host response without demonstrable persistence remains a separate possibility.

## 5. RUBV Remodels a Lysosomal Compartment

RUBV replication complexes have been demonstrated to be LAMP1-positive, acid-phosphatase-positive virus-modified lysosomes [8]. Earlier work extends the Rubella-lysosome lineage into the 1960s and 1970s, while later ultrastructural studies show that endocytosed extracellular cargo can enter RUBV replication vacuoles and that these structures communicate with the endocytic pathway and recruit rough ER, Golgi, and mitochondria. This places viral replication inside the same broad organelle system responsible for LDL-derived cholesteryl-ester processing, soluble lysosomal cargo delivery, and cholesterol export. It does not by itself show that LDL/LAL/NPC flux is abnormal.

## 6. The pH Convergence

SGMS1 separates acidification from membrane chemistry. SGMS1 is a multi-study RUBV dependency with an early sphingomyelin-dependent penetration role and a distinct late persistence phenotype. In SGMS1-knockout lipidomics, sphingomyelin falls while ceramide, CMH, CDH, Gb3, and GM3 rise; forced low-pH lipid mixing does not restore productive infection. Thus low pH and membrane composition are distinct requirements. For the NPC2 branch, the relevant host chemistry is pH + sphingomyelin → SMPD1/ASM → ceramide → BMP/LBPA → NPC2 transfer kinetics.

Purified human lysosomal acid lipase hydrolyzes cholesteryl esters under acidic lysosomal conditions [11]. NPC2-mediated cholesterol transfer also becomes more favorable under acidic late-endosomal/lysosomal conditions [12]. RUBV is pH-sensitive during entry, and established infection has been reported to perturb autophagic flux and acidic-vesicle biology [10]. More recent SGMS1 genetic work strengthens this intersection: loss of sphingomyelin synthase 1 impairs post-endocytic processes associated with endosomal acidification and also disrupts maintenance of persistent RUBV infection [26]. These findings do not prove that established RUBV replication compartments are alkaline. No calibrated direct measurement of CPV luminal pH has been identified. The stronger formulation is that RUBV biology intersects the machinery that establishes and uses acidic endolysosomal states, making direct compartment-specific pH measurement necessary.

## 8. Timing and Compartment Maturation Are Part of the Mechanism

The pH evidence contains real numerical anchors, but they describe different experimental stages and must not be collapsed into a single organelle-pH claim.

Väänänen and Kääriäinen reported direct RUBV low-pH membrane/fusion-related activity around pH 5.8. Katow and Sugiura later showed that brief exposure of RUBV-infected cells below pH 6 induced cell fusion, while exposure of virions around pH 5 produced irreversible E1/E2 conformational changes, altered infectivity and hemagglutination behavior, increased protease sensitivity, and promoted liposome binding. Mauracher and colleagues identified a RUBV capsid solubility/conformational transition between pH 5.0 and 5.5. Together, these studies establish a reproducible acidic RUBV chemistry staircase; they do not measure the luminal pH of an established replication compartment.

Human LAL supplies a second quantitative anchor. Physiological long-chain cholesteryl-ester and triglyceride hydrolysis is centered in deeper lysosomal acidity, broadly around pH 4-5 depending on substrate and preparation. Thus the historical RUBV triggering/fusion chemistry and LAL catalytic chemistry occupy overlapping but distinguishable acidic ranges. The separation is biochemically real; the unproven step is whether RUBV alters the maturation trajectory of an infected endolysosomal compartment enough to change LAL or NPC2 function.

The established-infection evidence points in the predicted direction but remains uncalibrated. In the five-day TO-336/SIRC system, infected cells showed a major loss of strongly acidic acridine-orange red vesicular signal. Continuous bafilomycin exposure was abandoned because of cytotoxicity, and the displayed experiment interrogated only the terminal 24 hours. Yagi's LysoPrime Green result cannot supply an absolute pH because LysoPrime is pH-resistant; Mori's chloroquine/bafilomycin experiments establish acidification dependence but likewise provide no luminal pH number. No calibrated absolute pH of an established RUBV-positive endosome, lysosome, autolysosome, or P150/dsRNA-positive CPV has been recovered.

Timing also matters to the trafficking hinge. Direct host work places Rab7 and VPS41/HOPS before ARL8 acquisition during endosome-to-lysosome maturation. Combined ARL8A/ARL8B loss produces the strongest cholesterol phenotype, whereas single-paralog loss can be buffered. This supports a specific RUBV prediction: P150-ARL8A engagement could be tolerated early and become functionally important only as the compartment matures and ARL8-dependent trafficking becomes more consequential. That timing model is a prediction, not a demonstrated RUBV mechanism.

The RUBV replication organelle is not a static compartment. Multiple RUBV programs show structural progression over time: early replication complexes appear, P150/RNA-positive vacuoles mature, and later 48-72-hour structures develop into large tubular or lamellar endolysosomal networks. Time-resolved ultrastructure also shows major reorganization of the cytopathic-vacuole, mitochondria, rough-ER, and Golgi architecture between earlier and later infection states. Different strains and cell systems can progress at different rates, so clock time alone is not sufficient; infection stage must be defined by both elapsed time and viral/compartment state.

This timing distinction changes how prior functional assays should be interpreted. Early low-pH and inhibitor studies primarily constrain entry, fusion, or early post-entry events. Oxford and Schild found that ammonium acetate and aminoadamantane had little or no antiviral effect when first added later than approximately 5 hours after infection, while pre-exposure followed by washout at inoculation did not inhibit growth. That result defines an early sensitive window; it does not measure the physiology of a mature RUBV-modified lysosome.

The established-infection acidification evidence also has a defined window. In the five-day TO-336/SIRC system, continuous bafilomycin exposure was abandoned because of cytotoxicity; the displayed experiment interrogated only the terminal 24 hours. Pásztor's established-infection phenotype therefore supports altered acidic-vesicle biology but does not define V-ATPase dependence across the entire five-day maturation sequence. Likewise, entry pH thresholds, hemagglutination-buffer pH, and virion conformational transitions do not measure the luminal pH of a mature P150/dsRNA-positive compartment.

The current model is explicitly stage-dependent: early entry/fusion -> establishment of P150/dsRNA replication structures -> endolysosomal maturation -> candidate ARL8/HOPS/CI-M6PR and membrane-chemistry divergence -> reduced NPC2 availability or transfer -> increased lysosomal free-cholesterol residence -> reduced ER arrival. Every arrow after structural maturation remains a prediction until measured in the same RUBV system.

This creates an important evidentiary rule for Paper II: a normal result obtained before the relevant compartment has matured cannot by itself falsify a phenotype assigned to established or late infection. Conversely, a late abnormality cannot be projected backward onto entry. The decisive experiment must follow the same pathway through time.

## 8. Cholesterol Throughput: Presence Does Not Equal Availability

The host pathway is LDL uptake -> LDL-derived cholesteryl ester -> LAL -> free cholesterol -> NPC2 -> NPC1 -> ER arrival and redistribution. The current lead model is post-LAL and NPC2-centered. It predicts that CE hydrolysis is substantially preserved, free cholesterol is generated, and the principal failure occurs when NPC2 availability or transfer becomes rate-limiting. Primary LAL failure and broader M6P-cargo failure remain explicit alternatives rather than co-equal lead hypotheses.

## 9. A Direct Viral Trafficking Foothold: P150N <-> ARL8A

The structural convergence is unusually tight. The ARL8A-positive P150N source clone maps to amino acids 1-463 of M33 RUBV, while independent localization work maps the functional P150 membrane-targeting/membrane-binding system to approximately amino acids 1-461, with amino acids 101-461 forming the mapped membrane-binding region and amino acids 1-100 required for proper function. The amino-acid 36-49 N-terminal helix is a critical targeting/maturation element; E36A, K46A, and I49A are high-priority interface-mapping mutants. No experiment yet shows that ARL8A binds this helix or mediates P150 membrane targeting.

A systematic RUBV-host interaction screen identified ARL8A among P150-associated host proteins. The discovery screen used a mixture of P150N and P150C, while the independent bead-based validation specifically used the P150 N-terminal construct [24]. ARL8 proteins regulate lysosomal positioning, transport, fusion, and recruitment of HOPS-related machinery. This makes the interaction unusually relevant to a virus whose replication complexes derive from lysosomes. The boundary is critical: physical binding does not prove that P150 inhibits ARL8A, changes ARL8 nucleotide state, disrupts ARL8B, displaces an effector, or impairs HOPS. The P150N-ARL8A interaction is therefore a direct viral foothold into the candidate trafficking system, not proof of the downstream model.

## 10. ARL8/HOPS/CI-M6PR/NPC2: The Direct Host Egress Mechanism

The cargo-delivery prediction extends beyond NPC2. Host M6P-deficiency proteomics places NPC2, SMPD1/acid sphingomyelinase, and FUCA1 among strongly depleted lysosomal cargo, while LAL has independent M6P-receptor-dependent uptake and functional CE-correction evidence. The resulting panel spans cholesterol transfer (NPC2), SM-to-ceramide chemistry (SMPD1), fucose-containing substrate turnover (FUCA1), and CE/TG hydrolysis (LAL). Coordinated lysosomal loss with increased extracellular pools would support a broader soluble-cargo routing lesion; selective NPC2 loss would support the current narrower lead model.

Acid-phosphatase-positive RUBV CPVs do not establish that this soluble-cargo system is intact. ACP2 can reach lysosomes through a substantially M6P-independent transmembrane route. A RUBV compartment could therefore remain LAMP1-positive and acid-phosphatase-positive while NPC2, LAL, SMPD1, or FUCA1 delivery is abnormal. This is a direct host routing principle; the corresponding RUBV cargo phenotype has not yet been measured.

Independent host-cell work shows that the BORC-ARL8-HOPS ensemble is required for lysosomal free-cholesterol egress [25]. Disruption of BORC, ARL8, VPS39, or VPS41 causes accumulation of free cholesterol in lysosomal structures, reduced downstream cholesteryl-ester storage, and ER cholesterol signaling consistent with reduced ER availability. The mechanism is particularly relevant because NPC1 localization remains relatively preserved while the soluble luminal transporter NPC2 is reduced in lysosomal compartments and increasingly secreted. CI-M6PR is depleted or degraded, consistent with defective receptor recycling and impaired delivery of M6P-tagged soluble lysosomal cargo [25]. LAL is also a mannose-6-phosphate-targeted soluble lysosomal hydrolase. Historical work shows that LAL can be secreted and recaptured through M6P-receptor-dependent uptake. Thus, if RUBV

functionally perturbs an ARL8/HOPS/CI-M6PR state, both sides of the LAL->NPC2 cholesterol route become experimentally relevant. No direct RUBV experiment has yet shown this.

## 11. RUBV Directly Engages Lipid and LAL-Adjacent Biology

Historical BHK21 measurements show why whole-cell cholesterol is an inadequate surrogate for this model. Bulk free cholesterol was nearly unchanged (5.6 control versus 5.8 RUBV) while phospholipids were extensively remodeled: LPC 4.8->2.4, sphingomyelin 5.3->4.5, PC 37.1->48.5, PS 7.7->5.8, PI 8.9->7.9, PE 25.0->27.2, and cardiolipin 6.5->3.8; the cholesterol:phospholipid ratio changed only from 0.17 to 0.19. These data support compartment-, species-, and flux-resolved measurements rather than a prediction of abnormal total cellular cholesterol.

The late RUBV-associated fucosylglyceride is also time-linked. It appears after approximately two days and was characterized around day four, was absent from uninfected controls and purified virions, represented roughly 1% of infected-cell lipid, contained approximately 40% sugar and 60% lipid with a fucose:glycerol ratio near 2:1, and had a strongly saturated fatty-acid profile. Its exact synthetic and degradative enzymes remain unknown. Its importance here is as direct evidence that late infection can acquire a chemically distinct lipid phenotype as the RUBV compartment matures.

Historical RUBV lipoprotein experiments add an upstream particle-state constraint. Native LDL and VLDL each produced RUBV hemagglutination-inhibition titers of 160; extracted LDL and VLDL lipid fractions retained activity at 128; apoLDL and apoVLDL fell to 15 and 10; and sonication increased activity to 480 and 640. Simple lecithin dispersions containing cholesteryl oleate, triolein, or cholesterol were each below 5. These results show that organized particle architecture and lipid/protein presentation materially affect the historical RUBV-facing phenotype. The assays used mildly acidic buffers around pH 6.0-6.2; those are assay conditions, not measurements of intracellular CPV pH.

Historical Lyon studies documented infection-associated lipid, phospholipid, fatty-acid, and glycolipid remodeling. Bardeletti and Voiland identified a unique late infection-associated glycolipid absent from uninfected controls and purified virus. The accessible chemical summary reports fucose and glycerol in a 2:1 molar ratio with palmitic, stearic, oleic, and myristic acids [27]. This is direct evidence that RUBV infection can generate a chemically distinct host-cell-associated lipid species; its exact structure remains unresolved. Geyer and colleagues profiled RUBV-infected primary endothelial cells. Re-analysis of the filtered supplementary data identifies a fetal endothelial probe annotated to LIPA/LOC100507575 at approximately 14.7-fold above control, together with sterol/lysosomal stress signals including CH25H and STARD5 [28]. The LIPA result is transcript evidence only. It does not prove increased LAL protein, lysosomal delivery, activity, CE hydrolysis, or egress. Indeed, a central prediction of this model is that transcript abundance could be dissociated from functional lysosomal availability.

## 12. Why Intracellular Cholesterol Availability Could Matter to Neurodevelopment

The developing nervous system depends on precisely localized lipids for membrane growth, axonal extension, synapse formation, receptor organization, myelination, and signaling. For neurodevelopment, total cholesterol is therefore an incomplete variable. A cell can contain abundant cholesterol while a distal axon, growing membrane, synapse, or ER compartment receives too little at the time it is needed.

## 13. Proposed Biological Subtype

If a RUBV-associated autism subtype exists, it should be prospectively identifiable by biology. The predicted signature is not 'Rubella exposure' alone. It is convergence between RUBV-linked virology or host response and a reproducible cellular phenotype involving endolysosomal maturation, NPC2-dependent cholesterol availability, membrane chemistry, or the independently testable glial/interferon route.

## 14. Relationship to MMR

This is not an MMR hypothesis. Congenital Rubella provides the human neurodevelopmental precedent. RA27/3 appears only because persistent infection with that Rubella strain has been demonstrated in particular immunodeficient hosts [7]. Population studies argue strongly against a universal or common MMR-mediated autism mechanism. A rare RUBV-associated subtype, if it exists, would have to be demonstrated directly. It cannot be inferred from autism diagnosis, vaccination status, or antibody titer.

## Four-Gate Endolysosomal Model

The endolysosomal route is not forced into a single lesion. Gate 1 is LDL input and recycling, including LDLR fate and RAB10/RAB1F/Ral-related routing. Gate 2 is M6P-cargo delivery through CI-M6PR/IGF2R,

RUFY1/retromer/GARP, and ARL8/HOPS, with NPC2, LAL, SMPD1, and FUCA1 as compartmental readouts. Gate 3 is deep lysosomal chemistry: pH, sphingomyelin, SMPD1/ceramide, PG/BMP, and NPC2 transfer. Gate 4 is post-NPC1 delivery through ORP1L/VAP/contact-site biology to ER/SOAT. The current lead is Gate 2/3 convergence at NPC2, but the experiment must distinguish all four.

RAB10, RABIF, and RALGAPB/Ral-related proteins are present in the broader RUBV host-interaction/search network and independently regulate LDLR fate or recycling in host biology. They justify measuring LDL input as an alternative branch; they do not establish a RUBV LDLR-routing phenotype.

## 15. Competing Explanations

- Historical congenital-Rubella autism observations may partly reflect broad fetal injury, sensory impairment, or intellectual disability rather than the specific lysosomal mechanism proposed here.
- Persistent RUBV may require immunodeficiency states absent from the proposed subgroup.
- Prolonged RUBV host response could be biologically relevant even when persistence is absent.
- RUBV may disturb acidic-vesicle biology without changing the pH of the replication compartment itself.
- P150N may bind ARL8A without functionally perturbing the ARL8/HOPS pathway.
- RUBV may remodel lipids without measurably changing LDL-derived cholesterol throughput.
- The LIPA-associated transcript signal may be compensatory, unrelated to lysosomal LAL availability, or partly attributable to the joint probe annotation.
- A cholesterol-flux phenotype could be a downstream marker of cellular stress rather than a causal contributor to neurodevelopment.
- Autism-like features in lysosomal or cholesterol disorders may reflect severe neurological disease and may not model idiopathic autism.

## 16. Falsification Criteria

- A negative assay performed only during entry or before the relevant P150/dsRNA-positive compartment has matured does not falsify the late NPC2/cholesterol-throughput prediction. The pathway must remain normal through established and late stages to count as a direct kill test.
- If stage-resolved measurements show normal ARL8/HOPS/CI-M6PR state, normal NPC2 lysosomal availability and transfer, normal membrane chemistry, and normal LDL-CE-to-ER flux throughout robust established and late RUBV replication, the endolysosomal branch is substantially falsified.
- Robust established RUBV replication with normal LAL delivery/activity, NPC2 routing, CI-M6PR state, lysosomal lipid composition, LDL-CE processing, lysosomal free-cholesterol residence, and ER arrival would substantially falsify the central cholesterol-routing branch.
- A P150-ARL8A interaction with no measurable effect on ARL8/HOPS effectors or downstream cargo routing would weaken the proposed trafficking hinge.
- No reproducible pH or maturation difference between RUBV-positive compartments and matched lysosomes would weaken the pH branch.
- A pH or membrane-lipid difference that does not correlate with LAL/NPC flux would argue that the change is a passenger rather than the bottleneck.
- Failure to identify a prospectively defined subgroup in which RUBV-associated measures converge with the predicted cellular phenotype would substantially weaken the disease-level hypothesis.
- Detection of RUBV without a linked cellular phenotype would not establish causation.

## 17. Experimental Roadmap

- The experiment should also test the quantitative pH staircase rather than a binary 'acidic/not acidic' state: calibrate absolute pH in P150/dsRNA-positive compartments at each maturation stage and compare that value with the historical RUBV triggering ranges (~5.8, below 6, ~5, and the 5.0-5.5 capsid transition) and the deeper LAL catalytic range (~4-5). The purpose is not to assume those values describe the same compartment; it is to determine whether the mature infected organelle follows a normal lysosomal acidification trajectory.
- At the same time points, measure NPC2 transfer function together with SM, ceramide, PG/BMP/LBPA, LAL activity, CI-M6PR retrieval, ARL8A/B membrane state, and LDL-CE -> FC -> ER/SOAT flux. The model predicts an ordered divergence after compartment establishment, not necessarily an abnormality at entry.

Phase 1 should establish the cellular phenotype as a time series rather than a single endpoint. Predefine early entry, 12-hour, 24-hour, 48-hour, 72-hour, five-day, and persistent-state sampling where the system permits, while indexing each sample by P150/dsRNA-positive compartment state rather than clock time alone. In the same cells, measure whether pH, ARL8 membrane recruitment, HOPS state, CI-M6PR routing, NPC2 availability/transfer, lysosomal free-cholesterol residence, and ER arrival diverge in a reproducible temporal order.

## 18. Integrated Thesis

The integrated hypothesis presented here is Jacob Boatman's model. Its disease-level prediction is that, in a susceptible biological subset, abnormal RUBV persistence, clearance, or host response may produce neurodevelopmentally relevant effects through two experimentally separable systems: a glial/interferon route and an endolysosomal trafficking/cholesterol-availability route. The updated endolysosomal branch is: RUBV-modified lysosome + P150N-ARL8A foothold -> possible ARL8-family/HOPS/CI-M6PR cargo-routing disturbance -> altered lysosomal delivery or retention of NPC2 and potentially LAL -> mismatch between LDL-derived lipid ingress, LAL processing, and post-LAL free-cholesterol egress -> increased lysosomal residence and reduced downstream cholesterol availability. Every functional arrow after the P150N-ARL8A interaction remains to be demonstrated directly in RUBV. The proposed neurodevelopmental consequence is altered intracellular cholesterol availability, not simple total-cholesterol deficiency. A cell can contain cholesterol while downstream axons, membranes, ER, or other compartments receive it at an inadequate rate. This model does not establish that RUBV causes autism generally, nor that a RUBV-associated subtype currently exists. It defines a subtype that should be discoverable or falsifiable. If the predicted virological and cellular signatures do not converge in a reproducible subgroup, the hypothesis should be abandoned or substantially revised.

## 19. The RUBV Autism Signal Is Longitudinal, Not Merely a Prevalence Number

The congenital-Rubella literature deserves more weight than a single 7% statistic. Chess's program followed children longitudinally and described autism as a dynamic syndrome within the congenital-Rubella population. Among 205 children reassessed around ages 8-9, two earlier autism diagnoses remitted and three new cases appeared. Her dedicated follow-up discussed recovery, chronicity, improvement, worsening, and delayed appearance of the autistic syndrome [2,3]. These observations were made under historical diagnostic criteria and in children with substantial sensory and neurological comorbidity. Their importance is nevertheless direct: RUBV-associated human neurodevelopmental pathology has included a longitudinal autistic phenotype rather than only intellectual disability or sensory impairment.

## 20. Delayed RUBV Neurological Disease: A Persistence Precedent Inside the CNS

Historical reports of progressive Rubella panencephalitis add a second human dimension. Patients with congenital Rubella later developed progressive neurological deterioration years after the original developmental infection. In one reported case, RUBV was isolated from brain tissue in a 12-year-old whose

progressive mental deterioration had begun at approximately eight years of age [16,17]. This is not an autism study. Its relevance is that RUBV can, under exceptional human circumstances, remain biologically relevant to delayed CNS disease long after the initiating infection. A contemporaneous 1975 correspondence asked whether delayed deterioration in progressive Rubella panencephalitis might have relevance to regression in language and intellectual performance observed in some children with autistic behavior [18]. That correspondence is historical hypothesis, not proof.

## 21. Bridge One: RUBV -> Glia -> Interferon -> Synaptic Development

The modern fetal-brain work gives this hypothesis a direct human neural entry point. RUBV predominantly infected microglia in human fetal brain tissue and generated strong interferon responses in neighboring neurons and neural progenitor cells [4]. Earlier work independently demonstrated astrocyte permissiveness [5]. These are not autism outcomes, but they show directly that RUBV can alter a developing human neural environment through glial infection and non-cell-autonomous signaling.

## 22. Bridge Two: Lysosomal Acidification -> Neuronal Connectivity -> Social Phenotype

NCOA7 has been implicated in human neurodevelopmental disorders and interacts with V-ATPase biology. Experimental Ncoa7 deficiency impairs lysosomal formation/function, alters neuronal development and neurite patterning, and reduces inhibitory synaptic contacts. Ncoa7-deficient mice display social-behavior abnormalities [15]. This does not show that acquired RUBV-mediated pH disturbance is equivalent to a genetic NCOA7 defect. It establishes that lysosomal proton-pump biology can propagate into neuronal connectivity and behavior, making direct pH measurement of RUBV-modified compartments worth performing.

## 23. Bridge Three: Sterol Biology Can Produce a Rigorously Defined Autism Phenotype

Smith-Lemli-Opitz syndrome (SLOS) is mechanistically distinct from the proposed RUBV pathway because it is a cholesterol-biosynthesis disorder rather than a lysosomal trafficking disorder. Its value here is specific: individuals with SLOS can meet formal autism criteria across reciprocal social interaction, communication, and restricted/repetitive behavior domains using standardized instruments [21]. This demonstrates that disturbed developmental sterol biology is capable of producing a rigorously defined autism phenotype.

## 24. Bridge Four: NPC Biology Is the Trafficking-Specific Neurodevelopmental Precedent

Niemann-Pick type C provides the closer mechanistic comparison because its core pathology is intracellular cholesterol trafficking. Pediatric NPC can initially present with features sufficiently autism-like to prompt ASD evaluation [22]. Such cases later develop the broader progressive neurological phenotype characteristic of NPC and therefore should not be treated as models of idiopathic autism. The cellular observation is more important: NPC1-deficient neurons can accumulate cholesterol in cell bodies and endolysosomal structures while distal axons contain less cholesterol [14]. The proposed RUBV model is not genetic NPC disease; it predicts a potentially acquired kinetic bottleneck that could partially reproduce the principle of compartmental cholesterol mismatch.

## 25. Bridge Five: Human Autistic Regression Intersects the SM-Ceramide Axis

A 2025 cerebrospinal-fluid lipidomics study reported broad sphingolipid differences in children characterized with autistic regression relative to neurodevelopmental and neurological control groups, including changes involving ceramides and sphingomyelins [23]. This is an association study. It does not establish lysosomal NPC2 dysfunction, RUBV involvement, or causation of regression. Its value is narrower: a human autism-associated phenotype can be accompanied by substantial disturbance of the same lipid family that controls NPC2 cholesterol transfer in defined biochemical systems.

The required next step is not to infer lysosomal membrane composition from CSF. It is to determine whether a prospectively defined RUBV cellular phenotype has an intracellular SM/BMP/ceramide signature and whether that signature predicts cholesterol-transfer rate.

## 26. Two Independent Mechanistic Routes May Converge

The model deliberately preserves two routes rather than forcing every observation into one linear chain.

Route A - glial/signaling: RUBV -> microglia/astrocytes -> interferon and neighboring neural-cell responses -> altered synaptic maturation/pruning -> autism-associated phenotype? Route B - endolysosomal/metabolic: RUBV -> virus-modified lysosome + P150N-ARL8A foothold -> possible ARL8/HOPS/CI-M6PR/NPC2/LAL cargo-routing disturbance + altered pH/membrane-lipid state -> cholesterol-throughput mismatch -> altered neuronal cholesterol availability -> neurodevelopmental phenotype? The routes could be independent, sequential, or mutually reinforcing. Current evidence does not establish their relationship. Their separation is a strength because failure of one does not automatically erase the other.

## 27. Stronger Subtype Hypothesis and Human Study Design

The case for directly testing a RUBV-associated autism subtype rests on convergence across independent evidence streams. Congenital RUBV already supplies the human autism precedent. Human fetal-brain studies supply direct glial and neighboring neural-cell biology. Persistence studies show that RUBV can remain biologically active in selected human contexts. RUBV directly remodels endolysosomal and lipid systems. Independent host experiments define an ARL8/HOPS/CI-M6PR/NPC2 mechanism capable of creating cholesterol presence without downstream availability.

Taken together, these findings make a direct subtype test scientifically warranted. They do not establish that a non-congenital RUBV-associated autism subtype already exists. The next evidentiary step is prospective convergence: establish the cellular RUBV signature first, predetermine the human criteria, and then ask whether that signature is enriched in a reproducible autism subgroup.

## 28. Experimental Expansion

- Make every mechanistic assay stage-aware. Pair clock time with compartment maturity and include early entry, establishment, 48-72-hour maturation, five-day established infection, and persistent states where feasible. A single early endpoint is not sufficient to test a late NPC2 model.

- Add synaptic and network endpoints to RUBV microglia-containing organoid experiments:

synapse density, inhibitory/excitatory balance, pruning, neuronal firing/network maturation, and neural-progenitor trajectories.

- Measure type-I interferon signaling by cell type and test selective IFNAR interruption as a rescue experiment.
- Measure calibrated pH specifically inside P150/dsRNA-positive, LAMP1-positive RUBV compartments.
- Measure LAL total/lysosomal/secreted protein and direct acid CE-hydrolase activity.
- Measure NPC2 lysosomal versus secreted abundance and CI-M6PR/MPR300/IGF2R localization/recycling.
- Measure ARL8A/ARL8B and VPS39/VPS41/HOPS state; map the P150N-ARL8A binding interface and test a binding-defective P150 mutant.
- Perform an LDL-CE pulse-chase measuring CE disappearance, free-cholesterol appearance/retention, and ER arrival.
- Quantify BMP/LBPA, sphingomyelin, ceramide, CMH, CDH/LacCer, GM3, Gb3, CE, free cholesterol, and TG in the RUBV-positive endolysosomal fraction.

- Determine whether pH, membrane-lipid, or cargo-routing changes predict neuronal/synaptic abnormalities rather than merely viral replication.
- Only after a reproducible cellular signature exists should a human autism cohort be prospectively stratified for that signature.

## 29. Interpretation Matrix

| Observed cellular pattern                                                                                           | Interpretation                                                                      |
|---------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| CE remains high; FC generation low; LAL activity low                                                                | Primary/strong LAL processing lesion.                                               |
| Lysosomal LAL falls while extracellular/secreted LAL rises; CE hydrolysis falls                                     | LAL ingress/delivery/retention defect.                                              |
| CE disappears; FC accumulates in lysosome; NPC2 lysosomal level/transfer falls or secretion rises; ER arrival falls | Egress-dominant ARL8/CI-M6PR/NPC2-side lesion; strongest fit to LAL mostly working. |
| LAL and NPC2 both misroute; CE and FC abnormalities coexist                                                         | Mixed soluble lysosomal cargo-routing defect.                                       |
| ARL8/HOPS/CI-M6PR/NPC2 state remains normal despite P150-ARL8A binding                                              | P150 interaction is not functionally driving the proposed trafficking hinge.        |
| All flux measures remain normal despite robust RUBV replication                                                     | Central cholesterol-routing branch substantially falsified.                         |

## 30. Publication Boundaries

- Do not convert the historical RUBV pH values (~5.8, below 6, ~5, or 5.0-5.5) into a claim that established RUBV CPVs have those luminal pH values. They are direct assay/fusion/conformational thresholds, not calibrated infected-organelle measurements.
- Do not describe LysoPrime Green as a numerical pH reporter. Its puncta differences are real microscopy observations, but the probe is pH-resistant and cannot establish pH <6 without a separate calibrated pH-sensitive measurement.
- Do not project a late established-infection phenotype backward onto entry unless the same variable has been measured across the intervening maturation sequence.
- Do not use an early-entry or pre-maturation negative assay to claim that the established/late NPC2 branch has been tested and excluded.
- Do not claim RUBV directly inhibits LAL, NPC2, NPC1, ARL8, HOPS, or CI-M6PR.
- Do not claim P150-ARL8A binding proves functional inhibition.
- Do not claim the LIPA/LOC100507575 microarray signal proves increased LAL protein or enzyme activity.
- Do not claim RUBV causes LAL or NPC2 secretion/misrouting until directly measured.
- Do not claim SGMS1-knockout lipidomics are infection-induced RUBV lipidomics.
- Do not claim the 1981 fucose:glycerol compound is a specific glycosphingolipid class that was not chemically demonstrated.
- Do not infer persistent RUBV from Rubella IgG titer alone.
- Do not infer a general or common MMR-autism mechanism from the congenital RUBV evidence or from this subtype hypothesis.
- Do not infer clinical treatment efficacy from this research model.
- Do not insert serotonin as a causal mechanism into the current Jake model.

### 31. Final Mechanistic Synchronization

The lead mechanism is also explicitly temporal. The predicted NPC2 defect is not assigned to the incoming virion or to the earliest entry compartment. It is assigned to the maturing RUBV-modified endolysosomal system. This matters because much of the historical functional literature samples entry or early post-entry biology, whereas the strongest model prediction belongs to established and late P150/dsRNA-positive compartments.

Within the endolysosomal route, LAL is not the lead bottleneck. NPC2 is. The lead-positive phenotype is substantial cholesteryl-ester hydrolysis and free-cholesterol generation followed by reduced lysosomal NPC2 availability or transfer, increased late-endosomal/lysosomal unesterified-free-cholesterol residence, and reduced ER arrival and SOAT re-esterification. Demonstrating this phenotype in RUBV would establish the cellular mechanism proposed in Route B; it would still require an independent human neurodevelopmental association before being assigned to an autism subtype.

The P150N construct used in the ARL8A interaction program maps to amino acids 1-463, almost exactly spanning the independently mapped N-terminal P150 membrane-targeting system. This strengthens the structural convergence at the viral trafficking foothold but does not prove functional ARL8A inhibition.

The missing same-system RUBV evidence remains explicit: functional P150/ARL8-to-HOPS/RUFY1/CIM6PR disruption, NPC2 lysosomal loss or secretion, impaired NPC2 transfer, and the complete LDL-CE -> LAL -> free cholesterol -> NPC2/NPC1 -> ER flux defect during established or late RUBV infection.

### Conclusion

The autism component of this hypothesis begins with direct human evidence. In congenital Rubella, autism was documented longitudinally, the phenotype changed over time, and Chess interpreted Rubella as etiologic in that population. The congenital record therefore establishes more than abstract plausibility: an autistic phenotype has occurred as part of human RUBV-associated neurodevelopmental disease.

Modern human fetal-brain studies add a second direct layer: RUBV infects developing glia and produces strong responses in neighboring neurons and neural progenitor cells. These findings do not establish a postnatal RUBV-autism mechanism, but they substantially narrow the biological gap between the historical congenital autism observation and a testable cellular pathway.

Paper II therefore proposes two separable routes worth direct testing. The glial/interferon route is anchored in developing-human-brain RUBV biology. The endolysosomal route is anchored in RUBV lysosomal replication, the P150N-ARL8A interaction, and a host NPC2 mechanism capable of producing cholesterol presence without availability. The decisive RUBV-specific functional bridge for Route B remains missing.

Timing is essential to both interpretation and falsification. RUBV compartments mature over time, and the current NPC2 model predicts a defect that emerges with established or late endolysosomal remodeling. Studies confined to entry or pre-maturation windows cannot be treated as direct tests of that late-stage mechanism. The critical experiment is longitudinal: follow the same trafficking, membrane, and cholesterol variables as the RUBV compartment matures.

The strongest conclusion presently justified is therefore specific. Congenital RUBV has a documented human autism precedent; a non-congenital RUBV-associated autism subtype remains unproven. It should be claimed only if RUBV-linked biology, a reproducible cellular or neural mechanism, temporal plausibility, and neurodevelopmental phenotype converge prospectively in the same subgroup. If that convergence is absent, the subtype hypothesis should be rejected or substantially revised.

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## SEPTEMBER 27, 2026 SYNCHRONIZATION ADDENDUM

Paper II remains the disease-level paper built on the congenital human RUBV-autism precedent and two separable mechanistic routes. This addendum synchronizes the endolysosomal route with Papers VI and VII. Where older Paper II wording assigns the lead bottleneck to NPC2, this later formulation controls. The congenital evidence, glial/interferon route, and requirement for a prospectively demonstrated non-congenital subtype remain unchanged.

### 32. CORRECTION TO ROUTE B: LAL-CENTERED BOTTLENECK #1

The current Jacob model no longer places the lead endolysosomal bottleneck at NPC2. Bottleneck #1 is LAL-centered lysosomal cholesteryl-ester/triglyceride throughput and capacity, with the proposed slow handoff localized after LAL hydrolysis and before NPC2 receives free cholesterol. NPC2 remains essential downstream transfer machinery and a competing discriminator.

Accordingly, Route B should now be read as a throughput hypothesis rather than an NPC2-deficiency hypothesis: RUBV-modified endolysosomal biology -> possible disturbance of cargo delivery, LAL-centered CE/TG processing and post-hydrolysis transfer -> altered free-cholesterol residence and downstream availability. Direct RUBV measurement of this complete flux remains missing. Neither the LIPA transcriptional signal nor any host/comparator result proves that RUBV directly inhibits LAL.

### 33. PAPER VI SYNCHRONIZATION: AUTISM, REGRESSION, MMR, AND TIMING

Paper VI sharpens the disease-level claim by specifying autism with developmental regression as a high-priority phenotype for testing the cholesterol-availability model. This does not convert associated human lipid findings into proof of RUBV causation. The disease-level hypothesis requires convergence in the same prospectively defined individuals: relevant RUBV biology, the predicted compartmental lipid/cholesterol state, neurodevelopmental phenotype, and the required temporal order.

Paper VI also makes timing a falsification criterion. Exposure must precede the proposed molecular lesion; the molecular lesion must precede or accompany the downstream lipid state; and that state must precede or accompany developmental loss. A phenotype present before the proposed exposure cannot be caused by that exposure, and a phenotype appearing only after regression may be consequence rather than cause.

The MMR extension remains separately constrained. Congenital and wild-type RUBV evidence does not establish that the attenuated rubella component of MMR produces the same cellular state. Existing population evidence cited in Paper VI weighs strongly against MMR as a common cause of autism. Any vaccine-strain extension therefore requires direct testing of the relevant attenuated rubella strain, identification of the proposed susceptible biology, and reconciliation with population evidence and chronology.

### 34. PAPER VII SYNCHRONIZATION: BOTTLENECK #2 - MAO

Paper VII adds a second experimentally separable bottleneck hypothesis at the mitochondrial outer membrane: monoamine oxidase (MAO). RUBV directly engages and functionally perturbs mitochondria through capsid-p32 interaction, mitochondrial-surface capsid, inhibition of mitochondrial precursor-protein import, reduced intramitochondrial p32, and selective respiratory-chain remodeling. Independently, MAO function is experimentally coupled to mitochondrial membrane lipid context.

Historical lipid-depletion and phospholipid-reconstitution experiments demonstrate strong A-form/B-form differences, while experimental mitochondrial cholesterol enrichment decreases MAO specific activity. A host bridge also exists: cholesterol-rich lysosomes form expanded STARD3-dependent contacts with mitochondria in NPC1-deficient or inhibited systems. These findings make the mitochondrial outer membrane a mechanistically credible convergence point, but no recovered experiment directly demonstrates RUBV-induced MAO-A or MAO-B dysfunction through membrane lipid remodeling.

MAO therefore becomes a third mechanistic branch to test alongside Paper II's existing Route A and Route B, not a replacement for either. Route A remains glial/interferon signaling. Route B remains endolysosomal/metabolic throughput. Route C is mitochondrial/MAO. The routes may be independent, sequential, or mutually reinforcing. Current evidence does not establish their relationship in autism.

### 35. UPDATED DISEASE-LEVEL TEST

A high-information human study should not select subjects by vaccination history, antibody titer, or autism diagnosis alone. It should prospectively phenotype developmental course, especially regression; measure the compartmental lipid/cholesterol signature predicted by Route B; and, where biologically feasible, include mitochondrial/MAO readouts predicted by Route C. Viral evidence and timing must be established independently rather than inferred from the downstream phenotype.

The strongest cellular bridge experiment should measure the LAL/post-LAL cholesterol state and MAO-A/MAO-B function in the same synchronized RUBV system. If the lysosomal phenotype appears without a mitochondrial/MAO phenotype, Route B can remain viable while Route C weakens. If MAO changes without the predicted lysosomal state, Paper VII's membrane bridge must be revised. If neither appears despite robust infection, the corresponding mechanistic branches fail.

### 36. UPDATED CONCLUSION BOUNDARY

The strongest conclusion remains bounded. Congenital RUBV provides a documented human autism precedent. Modern human neural studies establish direct RUBV access to developing glial and neighboring neural-cell biology. A non-congenital RUBV-associated autism subtype remains unproven and requires prospective convergence of virology, mechanism, chronology, and phenotype.

Papers VI and VII strengthen the testability of that hypothesis but do not convert it into an established general causal claim. Paper VI adds explicit regression, MMR, vaccine-strain, and temporal tests. Paper VII adds a mitochondrial/MAO hypothesis whose two sides are experimentally anchored but whose RUBV-specific functional bridge remains missing. Failure of any branch should narrow the model rather than be explained away.

### ADDITIONAL REFERENCES FOR THIS ADDENDUM

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